THE CHEMICAL INVESTIGATION OF SOME POISONOUS PLANTS IN THE N.O. SOLANACEÆ.

PART iii. THE OCCURRENCE OF NOR-HYOSCYAMINE IN *SOLANDRA LONGIFLORA.*

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In 1907, an investigation of the constituents of *Solandra lævis* Hook., (syn. *S. longiflora* Tussac), a plant belonging to the N.O. Solanaceæ, was carried out by the author,* when a new member of the group of midriatic alkaloids was discovered and isolated. The properties of this alkaloid were examined, and shown to differ from those of the solanaceous alkaloids previously known. It was accordingly named, from its source, "solandrine."

In 1912, Carr and Reynolds† published an account of their investigations of the alkaloids of another plant of the Order Solanaceæ, *Scopolia japonica;* and although it has been well known for the last thirty years, that this plant contained hyoscyamine, atropine, and scopolamine, these authors discovered a fourth alkaloid, which they isolated by means of a long and tedious process of fractional crystallisation. Carr and Reynolds named this new alkaloid nor-hyoscyamine, after determining its constitution and its relation to the other alkaloids. In addition, they proved the identity of their nor-hyoscyamine with the pseudo-hyoscyamine, which Merck in 1892 found in *Duboisia myoporoides*, which Hesse in 1901 found in *Mandragora officinarum*, but which neither of these eminent authorities was able to obtain in a pure state.

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* "Solandrine, a new Midriatic Alkaloid." Proc. Linn. Soc. N. S. Wales, 1907, xxxii., 789.

† Journ. Chem. Soc., ci., 1912, 946.

In the description of this nor-hyoscyamine base and its compounds, the author recognised a close resemblance to the properties of his "Solandrine," which he discovered five years earlier.

With interest thus renewed, a larger supply of material was obtained, and its alkaloids subjected to a more complete chemical investigation, the results of which have proved the identity of solandrine with Carr and Reynolds' nor-hyoscyamine.

Nor-hyoscyamine is the next lower homologue of hyoscyamine. In constitution it is a secondary base, and on the nitrogen bridge across the tropine radicle it contains the group $:N{\cdot}H$ in place of the tertiary group $:N{\cdot}CH_3$ in hyoscyamine. Only the *lævo*-rotatory forms of these occur in nature, and just as hyoscyamine may readily be racemised to inactive atropine, so the new alkaloid is converted into nor-atropine, the two pairs of isomers differing from one another in constitution only by a methyl group. It is this small difference, however, which constitutes the point of remarkable interest in this compound, since nearly all the known alkaloids are tertiary bases of the type $R_3 \vdots N$

Among the numerous chemical reactions in the metabolism of plants, one of the commonest, according to Pictet, is the methylation of amino groups by formaldehyde,

$$R:\overline{NH} + CH_2O = R:\overline{NCH_3} + O$$

These amino groups are numerous in the plant. Not only are they the products of katabolic processes, including the break-down of protein and chlorophyll, but they are also products of chemo-synthesis. Formaldehyde is formed by photo-synthesis in the leaves.

Further, it is well established that these methylated compounds can be made to undergo intramolecular change, whereby the methyl group is taken into the ring as an internal link in their chains of carbon atoms, and in this way such rings are enlarged, for example:—

pyrrole	methyl pyrrole	pyridine
$C_4H_4:\overline{NH}$	$C_4H_4:\overline{NCH_3}$	$\overline{C_5H_5} \vdots N$

Pictet* has isolated some of these simple substances from the leaves of a number of different plants, and he terms them proto-alkaloids. He believes the latter may form nearly all the complex alkaloids by methylation, condensation, and ring enlargement, as explained above.

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* Ber. der deut. chem. Ges., 40, 1907, 3771.

The nor-hyoscyamine molecule contains a reduced pyrrole and a pyridine ring united together; but the methylation of its nitrogen atom, which is found to have taken place in almost all the alkaloids, seems here to have been prevented. Only one other such case is known—the alkaloids of the hemlock, coniine and methylconiine—where there exist together, in the same plant, two alkaloids differing from one another only as secondary and tertiary bases, *i.e.*, with the amino group and its methyl derivative. Since, however, nor-hyoscyamine is always accompanied in plants by hyoscyamine, the methylation has been arrested only partially,—to a small extent in the case of nor-hyoscyamine, and to a very much larger amount in coniine.

OCCURRENCE OF NOR-HYOSCYAMINE.

Nor-hyoscyamine has been discovered in five distinct members of the Solanaceæ. Carr and Reynolds, by working on very large quantities of material (over 100 kilos.), have isolated this new base from the first four plants tabulated below. The *Duboisia myoporoides* was collected in the Philippine Islands, which is probably the northern limit of this Eastern-Australian tree. The *Mandragora* was investigated by Hesse.*

	Scopolia japonica.	*Datura metel.*	*Datura meteloides.*	*Duboisia myoporoides.*	*Mandragora officinarum.*
Nor-hyoscyamine	0·03%	0·01%	0·02%	0·15%	0·01%
L-hyoscyamine...	0·15	0·04	...	1·10	0·36
Atropine ...	...	...	0·03	...	...
Scopolamine ...	...	0·10	0·10	...	0·04
Meteloidine ...	...	...	0·05	...	...
Mandragorine ...	...	...	...	...	
air-dried	rhizome.	root, stem, leaf.		stem and leaf.	small amt. root.

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* Hesse, Journ. f. prakt. Chem., 64, 1901, 274.

Solandra longiflora.

This plant was first described by Tussac in the Flora Antillarum, 1818 (ii., 49, t.12). The name is synonymous with *S. lævis* of Hooker, which is described and figured in the Bot. Mag. 1848 (t.4345). Although the material for the first investigation was collected under the name of *S. lævis* Hook., the latter is now replaced by *longiflora*, since this has priority by thirty years.

S. longiflora is a beautiful evergreen shrub, grown in many gardens for its magnificent trumpet-flowers. It was originally introduced from the West Indies.

The material for this investigation consisted of the autumn prunings from a number of shrubs cultivated in the Botanic Gardens of Sydney: and the author takes this opportunity to express his appreciation of the kindness of the Director, Mr. J. H. Maiden, F.R.S., in providing the material in the quantity required, and in confirming the species.

EXPERIMENTAL.

Extraction of the alkaloid.—The air-dried leaves were ground to a fine powder, and exhausted with 70% alcohol by successive extractions during four weeks The sixth extract contained a trace only of alkaloid These alcoholic solutions, measuring about 40 litres, were distilled under diminished pressure, at a temperature not exceeding 40°C., till the residue was of the consistency of a thick syrup, and was free from alcohol. This dark brown mass was dissolved in hot water slightly acidulated, and filtered.

The fluid thus obtained was still dark brown in colour, quite opaque, and contained chlorophyll and fatty oils. All attempts to remove these by shaking out with petroleum spirit or ether were in vain, on account of the formation of emulsions. The aqueous extract was decolourised by agitation with a solution of gelatin, which was then removed by precipitation with alcohol. This yielded a clear yellow fluid from which the colour could not be removed with ether. The solution was next treated with basic lead acetate, and after filtering from the precipitate which formed, and taking out the lead, the alkaloids were separated by precipitation with Wagner's solution. From this precipitate the alkaloids were recovered in a clear water-white solution. This solution was extracted with ether until the latter ceased to dissolve any more impurities. By making the solution alkaline with ammonia, the alkaloids appeared as a white precipitate, and were dissolved out with chloroform. The chloroform solution was carefully dried with anhydrous sodium sulphate, and distilled under diminished pressure. A pale yellow viscous residue of alkaloids remained. This residue possessed a peculiar odour like pyridine, a characteristic which was also noted by Dunstan and others at the same stage in the isolation of their solanaceous alkaloids.

Formation of the Aurichlorides —The viscous residue was now dissolved in dilute hydrochloric acid, the solution filtered, and pure gold chloride added to it. The yellow precipitate, which first formed, was redissolved by addition of sufficient water, and by warming, then set aside to slowly crystallise. The spontaneous evaporation of the solution was continued so long as the bright yellow crystals formed, but towards the end, there separated viscous particles of a yellowish-brown colour, which readily melted on warming the liquid, and were completely soluble in alcohol. This uncrystallisable portion was decomposed and the gold removed, shaken out from alkaline solution with chloroform, and again treated with hydrochloric acid and gold chloride. In this way another small quantity of crystals was obtained, but the greater part still separated as viscous particles. By repeated attempts this could not be induced to crystallise further.

Fractional Crystallisation of the Aurichlorides.—The whole of the yellow crystals was brought into solution in one per cent. hydrochloric acid, and set aside to recrystallise slowly. The superfluid was decanted each day from its small crop of crystals. The melting-point of each crop was determined, and those portions having the same melting-points were combined. Each fraction was dissolved and crystallised many times, and by gradually bringing together similar fractions, each of these finally possessed a constant melting-point.

Results.—Extract (a) from 4 kgms. of dried leaves examined in 1911, yielded 2·63 gms. of aurichloride crystals, which were separated into the following fractions:—

(1) 1·32 gms. with melting-point 176°C.-178°C.
(2) 0·72 gm. with melting-point 165°C.
(3) 0·59 gm. with melting-point 137°C.-157°C.

Extract (b) from 2 kgms. of dried leaves examined in 1915, yielded 0·47 gm. of aurichloride salt, and this was separated into the following fractions:—

(1) 0·24 gm. with melting-point 176°C.
(2) 0·15 gm with melting-point 165°C.-166°C.
(3) 0·03 gm. with melting-point 137°C.-157°C.

The total weight of alkaloid, obtained as combined gold salts, thus falls short of the amount represented by the assay value of the leaves. The relative weights of the gold salts, on this account, do not indicate the relative amounts of each alkaloid in the plant, but only the amounts yielded in this case by the lengthy and elaborate process described.

PROPERTIES OF THE AURICHLORIDE FRACTIONS.

First Fraction.

Nor-hyoscyamine aurichloride.—The first fraction possessed the melting-point of Carr and Reynolds' nor-hyoscyamine salt, and the pseudo-hyoscyamine salt of Merck and Hesse; it was recrystallised a number of times, and the melting-point remained constant at 178°C. The crystals separated from dilute hydrochloric acid in brilliant golden-yellow scales. They contained no water of crystallisation.

In a weighed portion of this salt the amount of metallic gold was determined.

$$\text{Au} \quad 31{\cdot}94\% \text{ found.} \qquad \begin{cases} 32{\cdot}0\% \text{ required for nor-hyoscyamine.} \\ 31{\cdot}3\% \text{ required for hyoscyamine.} \end{cases}$$

Nor-hyoscyamine picrate.—The aurichloride was converted into picrate, by decomposition of the salt with sulphurous acid, and precipitation of the alkaloid with picric acid. The precipitate was redissolved in water, and crystallised several times, when a mass of pale yellow needle-shaped crystals was obtained, possessing the constant melting-point of 220°C, at which temperature the crystals decomposed. The melting-point of the picrate of Carr and Reynolds' nor-hyoscyamine, and also that of Merck's pseudo-hyoscyamine, was 220°C.

Nor-hyoscyamine oxalate.—This salt was prepared from the aurichloride. It crystallised from water and acetone in long, colourless, prismatic needles, which melted at 243-244°C. The melting-point of Carr and Reynolds' oxalate was 245°C.

Second Fraction.

Hyoscyamine.—The second fraction consisted of aurichloride crystals with melting-point 165°C. They were brilliant golden-yellow prisms, and were anhydrous.

A portion was converted into the picrate as before, and after two crystallisations, consisted of long, pale yellow needles, which melted at 163°C with decomposition.

Hyoscyamine aurichloride and picrate were prepared as controls from pure hyoscyamine, specially obtained from Messrs. Burroughs Wellcome's laboratories in London, through the kindness of the Sydney manager, Mr. Hector, and the author's best thanks are due to them. The melting-point of this pure hyoscyamine aurichloride was 165°C, and the picrate 163°C.

This second fraction, therefore, consists entirely of hyoscyamine.

Third Fraction.

Nor-atropine and atropine.—This fraction of the gold salts consisted of flaky yellow crystals, which were much paler in colour than the two previous fractions, and, unlike the latter, were not shining and glistening in appearance, but dull, opaque, and lustreless. From this, a small quantity of crystals was separated, which melted at 156-157°C. The nor-atropine of Carr and Reynolds is stated to have a melting-point of 157°C.

Another small fraction was obtained with a melting-point of 137°C. The melting-point of atropine gold salt is 137°C. This was checked by preparing the aurichloride from a sample of Messrs. Burroughs Wellcome's pure, optically inactive alkaloid.

The remainder of this third fraction was found to melt between 137° and 157°C, and was apparently a mixture of atropine and nor-atropine.

Many careful trials were made to isolate fractions melting above 178°C., from the mother-liquors of these aurichlorides; none, however, could be obtained, and it must therefore be concluded that the lævo and inactive scopolamines were absent.

Estimation of the total Alkaloids.

The modified method devised by Dunstan and Brown (Trans. Chem. Soc Lond , 75, 1899, 72) for the assay af alkaloids of the Solanaceous plants, was employed for the estimation of the total amount of alkaloids in the leaves.

The finely powdered material was extracted with alcohol, and the solution of the bases finally titrated with centinormal acid and iodeosin indicator.

Results.

(a) 1911 leaves—0·16% total alkaloids on plant dried at 100°C.

(b) 1916 leaves—0·17% total alkalaids on plant dried at 100°C.

Equivalent to 0·154% total alkaloids on air-dried leaves.

Equivalent to 0·033% total alkaloids on fresh leaves.

Alkaloids were detected also in extracts from the flowers, wood, and bark.

Summary

The leaves of *Solandra longiflora* are found to contain nor-hyoscyamine as the chief alkaloid. This was previously isolated and described by the author as a new alkaloid in 1907, under the name of "solandrine"; and is now identified with the alkaloid which Carr and Reynolds isolated in 1912, from other solanaceous plants. Solandra also contains hyoscyamine in lesser amount, and scopolamines are absent. The total amount of alkaloid obtained was 0·17% in the leaves (dried at 100°C).

In conclusion, the author desires to express his indebtedness to Professor Sir Thomas Anderson Stuart, for laboratory facilities afforded in carrying out this investigation.